

CLAIMS

What is claimed is:

1. A method of assessing the effects of a condition or a stimulus on translocation of a protein of interest from an intracellular location to the plasma membrane in
5 mammalian cells, comprising:
 - (a) culturing mammalian cells expressing modified protein of interest under a condition or a stimulus to be assessed for its effects on translocation of the protein of interest, wherein the modified protein of interest comprises the protein of interest and at least one epitope tag in an intracellular domain
10 and a fluorescent tag in an intracellular domain thereof, wherein the cells are referred to as test cells;
 - (b) determining a value corresponding to proportion of modified protein of interest at the cell membrane to total modified protein of interest in the test cells, thereby producing a test value;
 - 15 (c) comparing the test value with a control value, wherein the control value corresponds to the proportion of modified protein of interest at the cell membrane to total modified protein of interest in control cells, wherein the control cells are the same cells as are cultured in (a) and are cultured under the same conditions as in (a), except that the control cells are not cultured under
20 the condition or stimulus to be assessed, wherein if the test value is greater than the control value, translocation of the modified protein of interest has occurred.

2. A method of determining whether a protein of interest undergoes translocation from an intracellular location to the plasma membrane in mammalian cells in the presence of a condition or stimulator, comprising:
- (a) culturing cells that express modified protein of interest, wherein the
5 cells are cultured in the absence of the stimulator and the modified protein of interest comprises the protein of interest and at least one epitope tag in an extracellular domain and a fluorescent tag in an intracellular domain thereof, thereby producing cultured cells;
 - (b) determining the relative proportion of modified protein of interest at
10 the plasma membrane of cultured cells to total modified protein of interest in the cultured cells, thereby producing a control value;
 - (c) culturing cells that express the modified protein of interest under the same conditions as in (a) and in the presence of the stimulator, thereby producing test cells;
 - (d) determining the relative proportion of modified protein of interest at
15 the plasma membrane of test cells to total modified protein of interest in the test cells, thereby producing a test value; and
 - (e) comparing the control value and the test value, wherein a test value that is greater than the control value indicates that protein of interest
20 undergoes translocation following stimulation of the cells by the stimulator, thus determining whether the protein of interest undergoes translocation.
3. The method of claim 2, wherein an antibody which binds the at least one epitope tag and is labeled by a fluorescent tag which is detectable at a wavelength different from the wavelength at which the fluorescent tag of modified protein of
25 interest is detected is combined with the cultured cells produced in (a) and with the test cells produced in (c); the fluorescence of the fluorescent tag on the antibody and the fluorescence of the fluorescent tag of modified protein of interest are determined, wherein the intensity of fluorescence of the fluorescent tag on the

- antibody corresponds to protein of interest at the plasma membrane and the intensity of fluorescence of the fluorescent tag of modified protein of interest corresponds to total protein of interest in the cells; and the proportion of modified protein of interest at the plasma membrane to total modified protein of interest in cultured cells is compared with the proportion of modified protein of interest at the plasma membrane to total modified protein of interest in test cells, wherein if the proportion in test cells is greater than the proportion in cultured cells, translocation occurred.
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4. The method of claim 3, wherein the fluorescent tag on the antibody is phycoerythrin and the fluorescent tag of modified GLUT4 is green fluorescent protein.
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5. A method of identifying a drug which enhances translocation of a protein of interest from an intracellular location to the plasma membrane in mammalian cells, comprising:
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- (a) culturing cells which express modified protein of interest in the presence of a candidate drug, wherein the modified protein of interest comprises the protein of interest and at least one epitope tag in an extracellular domain and a fluorescent tag in an intracellular domain thereof, thereby producing cultured cells;
- 20
- (b) determining the relative proportion of modified protein of interest at the plasma membrane of cultured cells to total modified protein of interest in the cultured cells, thereby producing a test value; and
- (c) comparing the test value with a control value, which is the relative proportion of protein of interest at the plasma membrane to total protein of interest in cells cultured under the same conditions as the cultured cells of (a) but in the absence of the candidate drug, wherein if the test value is greater than the control value, the candidate drug is a drug which enhances
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translocation of the protein of interest from an intracellular location to the plasma membrane in mammalian cells.

6. A method of determining GLUT4 translocation from an intracellular location to the plasma membrane in mammalian cells, comprising:
 - 5 (a) culturing mammalian cells expressing modified GLUT4 protein under conditions which enhance or cause translocation of GLUT4, wherein the modified GLUT4 protein is GLUT4 protein comprising at least one epitope tag in an extracellular domain and a fluorescent tag in an intracellular domain thereof, wherein the cells are referred to as test cells;
 - 10 (b) determining the relative proportion of GLUT4 protein at the cell membrane to total GLUT4 protein in the test cells, thereby producing a test value;
 - (c) comparing the test value with a control value, wherein the control value is the relative proportion of modified GLUT4 at the cell membrane to
15 total modified GLUT4 in control cells, wherein the control cells are the same cells as are cultured in (a) and are cultured under the same conditions as in (a), except that the conditions do not enhance or cause translocation of GLUT4,
wherein if the test value is greater than the control value, translocation of
20 GLUT4 has occurred.
7. The method of claim 6, wherein the modified GLUT4 is expressed from a replication-deficient retrovirus encoding the modified GLUT4 protein.
8. The method of claim 6, wherein the modified GLUT4 is stably expressed by the test cells and the control cells.
- 25 9. The method of claim 8, wherein the modified GLUT4 protein is GLUT4 protein comprising seven myc epitope tags.

10. The method of claim 7, wherein the mammalian cells are selected from the group consisting of: adipocytes, fibroblasts and muscle cells.
11. The method of claim 10, wherein the cells are 3T3-L1 cells or Chinese Hamster Ovary cells, C2C12 cells and L6 cells.
- 5 12. A method of determining whether GLUT4 protein undergoes translocation from an intracellular location to the plasma membrane in cells in the presence of a stimulator, comprising:
 - 10 (a) culturing cells that express modified GLUT4 protein, wherein the cells are cultured in the absence of the stimulator and the modified GLUT4 protein is GLUT4 protein comprising at least one epitope tag in an extracellular domain and a fluorescent tag in an intracellular domain thereof, thereby producing cultured cells;
 - 15 (b) determining the relative proportion of modified GLUT4 protein at the plasma membrane of cultured cells to total modified GLUT4 protein in the cultured cells, thereby producing a control value;
 - (c) culturing cells that express the modified GLUT4 protein under the same conditions as in (a) and in the presence of the stimulator, thereby producing test cells; and
 - 20 (d) determining the relative proportion of modified GLUT4 protein at the plasma membrane of test cells to total modified GLUT4 protein in the test cells, thereby producing a test value.
13. The method of claim 12, wherein the modified GLUT4 protein is expressed by a replication-deficient retrovirus encoding the modified GLUT4 protein.
14. The method of claim 12, wherein the cells stably express modified GLUT4
25 protein.

15. The method of claim 13, wherein the modified GLUT4 protein is GLUT4 protein comprising at least one myc epitope tag and a fluorescent tag which is green fluorescent protein.
- 5 16. The method of claim 15, wherein the modified GLUT4 protein is GLUT4 protein comprising seven myc epitope tags.
17. The method of claim 16, wherein the cells are selected from the group consisting of: adipocytes, fibroblasts and muscle cells.
- 10 18. The method of claim 17, wherein the cells are selected from the group consisting of 3T3- L1 cells, Chinese Hamster Ovary cells, C2C12 cells and L6 cells.
19. The method of claim 13, wherein the replication-deficient retrovirus is pMX-GLUT4myc7-GFP.
- 15 20. The method of claim 15, wherein an antibody which binds the at least one epitope tag and is labeled by a fluorescent tag which is detectable at a wavelength different from the wavelength at which the fluorescent tag of modified GLUT4 is detected is combined with the cultured cells produced in (a) and with the test cells produced in (c); the fluorescence of the fluorescent tag on the antibody and the fluorescence of the fluorescent tag of modified GLUT4 are determined, wherein the intensity of fluorescence of the fluorescent tag on the antibody corresponds to GLUT4 at the plasma
20 membrane and the intensity of fluorescence of the fluorescent tag of modified GLUT4 corresponds to total GLUT4 in the cells; and the proportion of modified GLUT4 at the plasma membrane to total modified GLUT4 in cultured cells is compared with the proportion of modified GLUT4 at the

plasma membrane to total modified GLUT4 in test cells, wherein if the proportion in test cells is greater than the proportion in cultured cells, translocation occurred.

- 21 .The method of claim 20, wherein the fluorescent tag on the antibody is
5 phycoerythrin and the fluorescent tag of modified GLUT4 is green fluorescent protein.
- 22 The method of claim 12, wherein the stimulator is selected from the group
consisting of: insulin, decreased glucose concentration, high glucose
concentration, increased tumor necrosis factor- concentration, high
10 non-esterified fatty acid concentration, and other conditions which mimic insulin resistance.
23. A method of identifying a drug which enhances translocation of GLUT4 from
an intracellular location in the plasma membrane in mammalian cells,
comprising:
- 15 (a) culturing cells which express modified GLUT4 protein in the presence of a candidate drug, wherein the modified GLUT4 protein is GLUT4 protein comprising at least one epitope tag in an extracellular domain and a fluorescent tag in an intracellular domain thereof, thereby producing cultured cells;
- 20 (b) determining the relative proportion of modified GLUT4 protein at the plasma membrane of cultured cells to total modified GLUT4 protein in the cultured cells, thereby producing a test value; and
- (c) comparing the test value with a control value, which is the relative
proportion of GLUT4 protein at the plasma membrane to total GLUT4 protein
25 in cells cultured under the same conditions as the cultured cells of (a) but in the absence of the candidate drug,

wherein if the test value is greater than the control value, the candidate drug is a drug which enhances translocation of GLUT4 from an intracellular location to the plasma membrane in mammalian cells.

24. The method of claim 23, wherein the modified GLUT4 protein is expressed
5 by a replication-deficient retrovirus encoding the modified GLUT4 protein.
25. The method of claim 23, wherein the modified GLUT4 protein is stably expressed in the mammalian cells.
26. The method of claim 23, wherein the at least one epitope tag is a myc epitope tag and the fluorescent tag in the intracellular domain is green fluorescent
10 protein, blue fluorescent protein or red fluorescent protein.
27. The method of claim 26, wherein the modified GLUT4 protein is GLUT4 protein comprising seven myc epitope tags.
28. The method of claim 23, wherein the mammalian cells are selected from the group consisting of: adipocytes, fibroblasts and muscle cells.
- 15 29. The method of claim 28, wherein the cells are selected from the group consisting of: 3T3-L1 cells, Chinese Hamster Ovary cells, C2C12 cells and L6 cells.